

## Shrieking mice: Scientists in Monterey County make the world's first recordings of ultrasonic screams in wild California mice

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The old adage "quiet as a mouse" may be misleading. It turns out that mice are noisy creatures, filling the night air with howling sounds and high-pitched calls as they communicate with one another. The reason humans can't hear these nocturnal critters is because they vocalize in a frequency too high for the human ear to detect. And perhaps that's a good thing; otherwise, the sounds of howling mice would keep us awake all night long.

Researchers from the University of North Carolina at Greensboro recently made the world's first recordings of wild California mice (*Peromyscus californicus*) vocalizing in ultrasound. Though laboratory mice are known to communicate through ultrasonic calls, no one had previously documented this behavior in wild mice. The findings appeared this past April in the journal PLoS One.

It was serendipitous that Matina Kalcounis-Rueppell, biology professor at the UNCG, first discovered these calls in wild mice. She was doing field work for her Ph.D. dissertation at the time, studying California mice at Hastings Natural History Reserve in Monterey County's Santa Lucia

Mountains.

"I was live-trapping mice, sitting on the ground, when I got the sensation of sounds," said Kalcounis-Rueppell. Though the audible range for humans is from 20 hertz to 20 kilohertz (with frequencies above 20 kilohertz considered ultrasonic), mice vocalize at 18 kilohertz to 30 kilohertz. Because their lowest pitch is at the upper boundary of humans' audible range,

Kalcounis-Rueppell sensed she was hearing something.

"I thought I was hearing bats around me, but they seemed low to the ground. I thought it would be interesting to see if mice were making these sounds."

So she placed a bat detector (a device that converts the ultrasonic vocalizations of bats to a lower frequency that humans can hear) on the ground in an area she knew was occupied by California mice. Sure enough, on that first night she got ultrasound recordings of mouse calls. When slowed down by a factor of 22, these calls sound to the human ear like eerie, high-pitched howls.

"All around us there are animals that are communicating with each other through ultrasound and we are totally oblivious," said Kalcounis-Rueppell. "There is all this potential intercommunication going on in our basements, barns and backyards."

Previous studies have shown that white laboratory mice (a different species from California mice) use ultrasonic vocalization in many different contexts. When male lab mice are in the presence of female mice or their pheromones, they "sing," uttering repeated phrases with multiple syllables that, when

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slowed to frequencies humans can hear, sound similar to simple bird songs. Mouse pups cry out ultrasonically when they are separated from their mothers and these sounds will prompt female mice to retrieve them. Female mice also vocalize ultrasonically when their pups are taken away from them, calling for their babies to return.

Everything scientists know thus far about the context of this ultrasonic communication comes from laboratory experiments. The limitation of this is that, without comparing behavior of animals in a laboratory to that of animals in the wild, it is impossible to know if a particular behavior is simply an artifact of living in captivity.

This natural context is especially important to understand for ultrasonic vocalization in mice because this trait is often used by scientists studying mice as models for human behaviors, ranging from autism to addiction to parental care.

In particular, the California mouse is interesting to scientists because it is a monogamous species, with males and females mating for the entirety of their short lives. So this faithful mouse is often used as a laboratory model for studies looking at parental behavior, bonding between mates and monogamy.

"People have been looking at ultrasonic vocalization behavior in the lab to figure out the function of behavior," said Kalcounis-Rueppell. "If you understand the function of a behavior, you understand the behavior's benefit to the animal. The ultimate questions are about the evolution of a behavior. You really need to know what's going on in the wild ... before you can say anything of significance about an animal's behavior."

To validate previous laboratory studies, as well as allow her to ask interesting new questions about the

evolution of ultrasonic vocalization in mice, Kalcounis-Rueppell decided to move the microphones outside.

"People haven't been able to do this before because, no matter how sophisticated the equipment is, you have to know where the animal is living and aspects of their natural history to be able to isolate and record them," said Kalcounis-Rueppell. "We can do this at Hastings because we've been studying them for so long there that we know a lot about their natural history."

With the help of graduate students Jessica Briggs and Radmila Petric, she made the trek back to Hastings, where she could continue the research project in the 2,500-acre ecosystem managed by the University of California Natural Reserve System.

The first step was to trap each study mouse in a live-trap, give it an individual ear tag before releasing it, then recapture individuals over a season to define their territory. Researchers then placed an array of microphones capable of recording ultrasound in the habitat where they knew the mice were living. In addition, they fitted each mouse with a tiny radio transmitter that it wore like a necklace. Each radio transmitter emitted a unique frequency so researchers could determine the location of a specific mouse. This allowed scientists to determine which individual was vocalizing — an important method of ruling out calls from other mouse species. To rule out that a mouse not fitted with a transmitter could be recorded, researchers mounted a thermal camera in the tree canopy so they could see the mice running around while they were eavesdropping on their "conversation."

Once mice were recorded, the vocalizations were analyzed using computer software that displays the sound waves visually in a spectrograph, a graph

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showing frequency over time. The next step was to record California mice in captivity at the Peromyscus Genetic Stock Center at the University of South Carolina in Columbia and compare the sounds with those in the wild.

"We found that the types of calls made in captivity and in the wild are generally very similar," said Kalcounis-Rueppell. "This shows that sounds made in the lab are not an artifact of the lab. This is important because it shows that the work we do in the lab is validated — for the first time we know that mice in the wild also make these sounds."

There were also some differences in recordings of wild mice versus captive mice.

"The vocalizations recorded in wild mice have a greater range in frequency. They may be slightly higher or lower in pitch or slightly longer in duration than those you find in the lab," said Kalcounis-Rueppell. "The mice in the lab sound more similar to each other than mice in the wild."

Though the reason for these differences is unknown, one possible answer is that animals in the wild may adjust their pitch relative to background noises. Still, despite variability in wild California mouse calls, this work validates previous ultrasound work in the lab because the majority of studies looked at rates of sound, rather than characteristics of sound.

"Now we can begin to answer interesting questions about evolutionary function of these vocalizations in wild mice," said Kalcounis-Rueppell. "We can look at behaviors in the lab and make hypotheses about why those behaviors exist. However, until we see these behaviors in nature, we can't really answer questions about the evolution of those behaviors and why they're beneficial to individuals of that

species."

This study, including links to audio files of mouse vocalizations, is available at <http://dx.plos.org/10.1371/journal.pone.0009705>

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